Effect of External or Internal Fecal Contamination on Numbers of Bacteria on Prechilled Broiler Carcasses[1]

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ABSTRACT During processing, fecal material may contact broiler carcasses externally or internally. A study was conducted to determine the effect of external vs. internal fecal contamination on numbers of bacteria on broiler carcasses. In each of 3 trials, 12 carcasses just prior to evisceration were obtained from a commercial processing plant, placed on a shackle line, and eviscerated with commercial equipment in a pilot scale processing plant. Also, approximately 20 intestinal tracts were collected from the processing plant; then cecal contents were collected and pooled. One gram of cecal content was placed on the exterior breast skin (external), inside the carcass cavity (internal), or not applied (control). All carcasses were held 10 min, then placed on the shackle line and passed through a commercial inside-outside bird washer set at 552 kPa, 5 s dwell time, using approximately 189 L per min of tap water at ambient temperature. After a 1-min drip, whole carcass rinses were conducted on each carcass, and coliforms, *Escherichia coli,* and *Campylobacter* counts were determined and reported as log cfu/mL of rinse. External carcass contamination resulted in significantly higher ($P < 0.05$) coliform, *E. coli,* and *Campylobacter* numbers than internal contamination (5.0 vs. 4.5, 4.9 vs. 4.2, and 3.6 vs. 2.6, respectively). Control carcass counts were significantly lower than external or internal carcass contamination counts for coliforms (3.7), *E. coli* (3.6), and *Campylobacter* (2.2). External contamination resulted in higher numbers of bacteria after carcass washing, but carcasses with internal contamination still have higher numbers of bacteria after washing than carcasses without applied contamination.

Key words: fecal contamination, inside-outside bird washer, coliform, *Escherichia coli, Campylobacter*

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INTRODUCTION

The USDA's Food Safety Inspection Service (**FSIS**) implemented *Escherichia coli* and *Salmonella* testing on postchill poultry carcasses to reduce or eliminate bacterial pathogens (USDA, 1996). To assist this effort, FSIS mandated a zero tolerance policy for fecal material on poultry carcasses prior to entering the chiller (USDA, 2005). These actions demonstrate FSIS is determined to reduce fecal contamination and associated numbers of pathogens on processed poultry.

Fecal material or ingesta, and bacteria associated with these contaminants, may be introduced to the broiler carcass during processing. Damage to the intestines may occur during evisceration process, leading to carcass contamination (Sams, 2001). Russell (2003) reported that intestines cut during the evisceration process ranged from 2 to 34% of broilers evaluated in 1 processing plant. At a commercial processing plant, 25% of crops collected at the cropper machine were observed to have been damaged (Hargis et al., 1995). Even the presumably gentler version of manual crop removal resulted in an overall average of 22% ruptured broiler crops (Buhr and Dickens, 2001).

Little or no data are available describing the location of specific types of contamination on broiler carcasses. One study reported visible contamination levels on or in broiler carcasses processed by 2 different evisceration systems at 2 different plants. Unfortunately, contamination was defined as combined feces, ingesta, and bile defects (Russell and Walker, 1997). In that study, postevisceration external carcass contamination ranged from 8.4 to 25.2% and internal contamination from 4 to 28% at the first plant, depending on type of evisceration system; external contamination rates at the other plant ranged from 5.5 to 9.2%, and internal rates ranged from 6.6 to 7.5%, depending on the evisceration system. Russell (2003) collected data on visible external and internal fecal contamination for 1,000 broiler carcasses, but the published results consisted of only the total combined percentages of contamination.

There is evidence that bacterial numbers differ between external and internal areas of the carcass. Bodnaruk et al. (1998) reported that *E. coli* numbers recovered from the

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internal cavity of turkey carcasses were not different from the external breast skin area. However, these areas contained lower *E. coli* numbers than the external thigh or back skin areas. Lillard (1991) found no difference between the external and internal prechill carcass surface for total aerobes, although the internal cavity had higher numbers of *Enterobacteriaceae* than the external surface.

Processors have incorporated inside-outside bird washers (**IOBW**) in an attempt to reduce visible contamination and bacterial numbers on carcasses. Generally, IOBW are able to reduce visible contamination on carcasses (Fletcher et al., 1997; Jimenez et al., 2003). Reports on the effect of IOBW on carcass microbiology have been mixed. Some research has shown a reduction in carcass bacterial numbers when using carcass washers or IOBW (Keel and Parmalee, 1968; May, 1974; Izat et al., 1988). However, others have reported little effect of washing on numbers or incidence of bacteria on carcasses (Thomson et al., 1974; Yang et al., 1998; Northcutt et al., 2003). Smith et al. (2005) found that an IOBW reduced the incidence of *Campylobacter* and *Salmonella* on visibly contaminated carcasses, but Bashor et al. (2004) did not find a reduction in *Campylobacter* incidence after carcasses were washed in IOBW in commercial plants.

Commercial processing, particularly the evisceration process, may result in carcass contamination. There are reported differences in visible contamination and bacterial numbers between the external surface and internal cavity. The IOBW is utilized commercially as a last step for reducing visible contamination and bacterial populations on prechill carcasses, yet research reports differ regarding its efficacy. Therefore, the purpose of this study was to determine the effect of external or internal fecal contamination on the numbers and incidence of coliforms, *E. coli,* and *Campylobacter* after evisceration and passage through an IOBW.

MATERIALS AND METHODS

In each of 3 replicate trials, 12 broiler carcasses were obtained from a processor at the shackle line transfer point just prior to evisceration. Carcasses were individually bagged and immediately transported to a poultry processing pilot plant. Carcasses were hung on a shackle line and eviscerated with commercial equipment. A fecal contaminant, prepared as described below (1.0 g of cecal contents), was applied to the breast skin surface (external), the medial surface of the sternum (internal), or not applied (control). All carcasses were then left uncovered at room temperature for 10 min to simulate the maximum time carcasses would typically be in contact with fecal contamination resulting during evisceration (approximating the time from the beginning of evisceration to final carcass washing). Carcasses were passed through a commercial IOBW set at a line speed of 140 birds per min, water pressure at 552 kPa, with a dwell time of approximately 5 s. Water usage for the IOBW was approximately 189 L/min. Carcasses were spaced on the shackle line with empty shackles between to prevent potential spray cross-contamination. After dripping for 1 min, carcasses were placed in clean plastic bags to conduct a whole carcass rinse procedure (**WCR**). Two hundred milliliters of 0.1% peptone was added, and carcass bags were shaken for 1 min in an automated mechanical shaker. Rinsates were aseptically collected and prepared for serial dilutions in 0.1% peptone.

The fecal contaminant was prepared from cecal contents from approximately 20 intestinal tracts collected at the processing plant concurrent with carcass collection. Cecal contents were pooled then stirred manually with a sanitized spatula. Samples of this mixture were taken, and coliforms, *E. coli,* and *Campylobacter* numbers were determined as described below.

Coliforms and *E. coli* were enumerated by plating 1 mL from a serial dilution of the sample onto duplicate Petrifilm *E. coli*/coliform count plates (3M Health Care, St. Paul, MN). Petrifilm plates were incubated at 35°C for 18 to 24 h, and the types of colonies characteristic of coliforms and *E. coli* were counted. *Campylobacter* culture was conducted by direct plating onto the surface of Campy-Cefex agar (Stern et al., 1992), which was then incubated for 48 h in a microaerophilic atmosphere consisting of 5% O_2, 10% CO_2, and balance N_2 (BOC Gases, Chattanooga, TN). Colonies with the characteristic appearance of *Campylobacter* were counted. Each colony type from every sample was confirmed as *Campylobacter* by observation of cellular morphology and motility on a wet mount using a phase contrast microscope. Each colony type was further confirmed by a positive reaction from a serological latex agglutination test kit (Panbio Inc., Columbia, MD).

Coliform, *E. coli,* and *Campylobacter* numbers were converted to log cfu/mL of rinsate for statistical analysis. Differences between treatments (external, internal, or control) were tested by ANOVA using GLM procedures of SAS (SAS Institute, 1999). Data were pooled across trials because there was not a significant trial × treatment interaction, except for *Campylobacter* numbers in trial 2, which was due to low recovery rates resulting from low numbers of *Campylobacter* in the ceca on that particular day. Means were separated by Tukey's method in SAS. Numbers of bacteria for a sample in trial 2 that was lost resulted in those numbers treated as missing values in the analysis. Nondetectable numbers of *Campylobacter* for 5 carcass samples in trial 2 were assigned the minimum detection limit value of 0.1 log cfu/mL of rinsate.

RESULTS AND DISCUSSION

The fecal contaminant prepared from pooled cecal contents from all 3 trials contained an average of 5.7 coliforms, 5.9 *E. coli,* and 4.3 *Campylobacter* log cfu/g. The cecal material in trial 2, however, contained only 2.8 log cfu/g of *Campylobacter.* This resulted in a significant trial × treatment interaction in trial 2 for *Campylobacter* numbers as a number of postwash carcass values were below the detection limit.

Table 1. Mean numbers (log cfu/mL of rinsate) ± SD and incidence (number of positive carcasses/number of carcasses sampled) of coliforms, *Escherichia coli*, and *Campylobacter* from prechilled broiler carcasses with external or internal fecal contamination (1.0 g) applied prior to the inside-outside bird washer

Contamination	Coliforms	*Escherichia coli*	*Campylobacter*
	log cfu/mL of rinsate		
External	5.0^{a} ± 0.1 (11/11)	4.9^{a} ± 0.2 (11/11)	3.6^{a} ± 0.5 (11/11)
Internal	4.5^{b} ± 0.2 (12/12)	4.2^{b} ± 0.2 (12/12)	2.6^{b} ± 0.6 (8/12)
None (control)	3.7^{c} ± 0.2 (12/12)	3.6^{c} ± 0.2 (12/12)	2.2^{c} ± 0.4 (11/12)

[a–c]Within columns, means lacking a common superscript differ significantly ($P < 0.05$).

Mean numbers of coliforms, *E. coli*, and *Campylobacter* of prechill (post-IOBW) broiler carcasses with external or internal fecal contamination are presented in Table 1. Carcasses with external contamination had a coliform count of 5.0 (log cfu/mL of rinsate), which was significantly higher ($P < 0.05$) than the internal contamination count (4.5), which was higher than counts for control carcasses (3.7). External contamination produced the highest *E. coli* counts, with significantly lower counts recorded for internal contamination, with the lowest counts found for control carcasses (4.9, 4.2, and 3.6 log cfu/mL of rinsate, respectively). Numbers of *Campylobacter* for external contamination averaged 3.6 log cfu/mL of rinsate, which were significantly higher than internal contamination (2.6), which were higher than control counts (2.2).

Previous research has shown that numbers of *Enterobacteriaceae* recovered by rinsing prechill broiler carcasses differed significantly between the internal cavity, 5.1 log cfu/carcass, and the external surface, 4.8 log cfu/carcass (Lillard, 1991). Bodnaruk et al. (1998) reported differences in *E. coli* numbers for turkey carcasses swabs from the internal cavity and specific external skin areas. The numbers for the cavity and external breast surface were not different from each other (2.7 and 2.6 log cfu/cm^2, respectively), but both were lower than the thigh or back external surfaces (6.7 and 7.6 log cfu/cm^2, respectively). Results from the present study support previous findings that numbers of bacteria may differ between the internal cavity and external surface.

Incidence of coliforms and *E. coli* was 100% for all carcasses in the study regardless of treatment. *Campylobacter* incidence for external contamination treatment was 100%, whereas internal was 67% (8/12), and control was 92% (11/12). Several carcasses in trial 2 were below the detection limit for *Campylobacter* because of low numbers in the cecal contents that were applied, which resulted in a seemingly lower incidence of *Campylobacter*. One previous report was found regarding pathogen incidence on the exterior surface and interior cavity of broilers. Lillard (1991) reported that *Salmonella* incidence of broiler carcasses was lower (8.6%) when only the body cavity was rinsed, whereas rinsing the external surfaces resulted in a higher incidence (16.1%).

The difference in bacterial numbers between external and internal contamination treatments leads to several questions regarding possible explanations of the results. First, does contamination adhere less readily to internal vs. external surfaces; secondly, does the IOBW remove internal contamination more easily than external contamination; thirdly, is the WCR technique incapable of adequately sampling carcasses with internal contamination; or, finally, is a combination of these factors responsible for the results?

Jiminez et al. (2002) postulated that bacteria introduced to the carcass during evisceration (from fecal contamination) are loosely attached to the skin surface because the attachment process is time dependent. They also stated that the IOBW may decrease pathogen incidence among carcasses without visible contamination but could increase the incidence of contamination when visibly contaminated carcasses were washed adjacent to other carcasses via cross contamination. However, Smith et al. (2005) did not find evidence that carcasses with visible fecal contamination cross contaminated other carcasses within the IOBW. In the present study, carcasses with external contamination were occasionally observed with fecal residue remaining after the IOBW, but no contaminant residue was observed inside carcasses with internal contamination after the IOBW. The premise that attachment is time dependent could be expanded to include the idea that bacterial attachment is also tissue dependent as well as time dependent. The medial surface of the sternum inside the thoracic cavity has, subjectively, a smooth nonporous texture and appearance. This could explain why carcasses with fresh internal contamination had lower numbers of bacteria and also no remaining visible contamination after washing. However, Lillard (1991) concluded that because bacteria were removed from the internal cavity at the same rate as external surfaces by rinsing, the 2 areas appeared equal for bacterial adhesion. All of the carcasses in that study were presumably free of visible (and fresh) contamination, so those results are not necessarily reconcilable with the present study.

The IOBW may provide a better washing action on the enclosed inner surfaces than the exterior surfaces, depending on factors such as the total amount of water used, total surface area washed, the pressure of the water, and nozzle direction and spray pattern. However, no direct research is available to determine external vs. internal cleaning efficacy of the IOBW. Mulder and Bolder (1981)

surveyed several commercial plants using spray cabinets (no internal washing) and IOBW. They found IOBW offered no advantage over external spray cabinets for reducing total bacteria and *Enterobacteriaceae*, although a direct comparison of the spray washers vs. IOBW was not conducted.

The WCR method is widely used for enumerating total poultry carcass bacteria and is required by USDA to determine enumeration of *E. coli* and incidence of *Salmonella* in poultry plants (USDA, 1996). However, research on the ability of the WCR method to fully recover bacteria in the carcass cavity was not found. Although more friction is produced on the exterior of the carcass during rinsing via contact with the bag than is available on the interior, it seems unlikely that, due to the nature of the excess liquid and shaking of the carcass in all directions, substantial numbers of bacteria in the cavity would be missed. Therefore, the WCR appears to be an appropriate method for adequately sampling the internal cavity and exterior surface of broiler carcasses.

After the IOBW, carcasses with internal contamination had fewer numbers of coliforms and *E. coli*, from 0.5 to 0.7 log cfu/mL, respectively, than carcasses with external contamination. The internal carcasses were also 1.0 log cfu/mL lower for *Campylobacter* than external carcasses. Potential improvement in IOBW operation should be directed toward cleaning of the external surface of the carcass. Also, questions regarding the cause of the difference in bacterial numbers between carcasses with external vs. internal contamination have not been resolved, and this area will require more research.

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